Enclosure to letter dated 15 November 2005 concerning European Patent Appln. No. PCT/EP2005/000555; - DSM IP Assets B.V. -; ref:21726W0, clean version

REVISED CLAIMS

1. Process for the biochemical synthesis of 6-amino caproic acid wherein either 6-aminohex-2-enoic acid of formula [1] (6-AHEA)

$$H_2N - CH_2 - CH_2 - CH_2 - CH = CH - COOH \qquad [1]$$

or wherein 6-amino-2-hydroxy-hexanoic acid (6-AHHA), a compound capable of being transformed into 6-aminohex-2-enoic acid, is treated with an enzyme having α,β-enoate reductase activity towards molecules containing an α,β-enoate group and a primary amino group, in particular with an enzyme having α,β-enoate reductase activity towards 6-aminohex-2-enoic acid.

2. Process according to claim 1, characterized in that the enzyme having α,β-enoate reductase activity is an enzyme originating from a microorganism from the group of species of *Acetobacterium* sp., *Acremonium* sp., *Agrobacterium* sp., *Burkholderia* sp., *Cephalosporium* sp., *Clostridium* sp., *Escherichia* sp., *Moorella* sp., *Ochrobactrum* sp., *Pseudomonas* sp., *Salmonella* sp., *Shigella* sp., *Tilachlidium* sp., *Yersinia* sp., and *Vibrio* sp.

3. Process according to one of claims 1 or 2, characterized in that the enzyme having α,β-enoate reductase activity is an enzyme originating from *Acremonium* sp., *Clostridium* sp., *Moorella* sp. or *Ochrobactrum* sp.

4. Process according to claim 3, characterized in that the enzyme having is an enzyme from *Acremonium strictum* CBS114157, *Clostridium tyrobutyricum* DSM1460, *Moorella thermoacetica* DSM1974, *Ochrobactrum anthropi* NCIMB41200, or *Clostridium kluyveri* DSM555.

5. Process according to claim 1 or 2, characterized in that the enzyme having α,β-enoate reductase activity has aerostable α,β-enoate reductase activity and is an enzyme originating from a microorganism from the group of species of *Agrobacterium* sp., *Burkholderia* sp., *Escherichia* sp., *Pseudomonas* sp., *Salmonella* sp., *Shigella* sp., *Yersinia* sp., and *Vibrio* sp.

6. Process according to claim 5, characterized in that the enzyme having aerostable α,β-enoate reductase activity is an enzyme originating from an *Escherichia coli* species.

7. Process according to claim 6, characterized in that the enzyme having aerostable α,β-enoate reductase activity is an enzyme originating from from *Escherichia coli* K12.

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8. Process according to any of claims 1-7, characterized in that 6-aminohex-2-enoic acid is being converted into 6-amino caproic acid at a pH in the range of from 3 to 9.

9. Process according to claim 8, characterized in that, the pH is in the range of from 4 to 8.

10. Process according to claim 9, characterized in that the pH is in the range of from 5 to 8.

11. Process according to claim 8, characterized in that, the pH is in the range of from 5.5 to 7 under anaerobic conditions and of from 6.5 to 8 under aerobic conditions.

12. Process according to any of claims 1-11, characterized in that the process is carried out in a host organism selected from the group of genera consisting of *Aspergillus*, *Bacillus*, *Corynebacterium*, *Escherichia* and *Pichia*.

13. Process according to claim 12, characterized in that the process is carried out in a host organism selected from the group of *Escherichia coli*, *Bacillus*, *Corynebacterium glutamicum*, *Aspergillus niger* or *Pichia pastoris* host organisms.

14. Process according to claim 12 or 13, characterized in that in the host organism an α,β-enoate reductase gene encoding an enzyme having α,β-enoate reductase activity towards molecules containing an α,β-enoate group and a primary amino group is cloned and expressed.

15. An *Escherichia coli* host cell wherein the α,β-enoate reductase gene from *Ochrobactrum anthropi* NCIMB41200, or from *Acremonium strictum* CBS114157 is cloned and expressed.

16. A *Bacillus* host cell wherein the α,β-enoate reductase gene from *Moorella thermoacetica* DSM1974, or from *Clostridium tyrobutyricum* DSM1460, or from *Ochrobactrum anthropi* NCIMB41200, or from *Acremonium strictum* CBS114157 is cloned and expressed.

17. A *Corynebacterium glutamicum* host cell wherein the α,β-enoate reductase gene from *Moorella thermoacetica* DSM1974, or from *Clostridium tyrobutyricum* DSM1460, or from *Ochrobactrum anthropi* NCIMB41200, or from *Acremonium strictum* CBS114157 is cloned and expressed.

18. An *Aspergillus niger* host cell wherein the α,β-enoate reductase gene from *Acremonium strictum* CBS114157, or from *Moorella thermoacetica* DSM1974, or from *Clostridium tyrobutyricum* DSM1460, or from *Ochrobactrum anthropi* NCIMB41200 is cloned and expressed.

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19. A *Pichia pastoris* host cell wherein the α,β-enoate reductase gene from *Acremonium strictum* CBS114157, or from *Moorella thermoacetica* DSM1974, or from *Clostridium tyrobutyricum* DSM1460, or from *Ochrobactrum anthropi* NCIMB41200 is cloned and expressed.

20. A host cell selected from the group of *Aspergillus*, *Bacillus*, *Corynebacterium*, and *Pichia* host cells, in which the aerostable α,β-enoate reductase gene *nemA* from *E. coli* K12 is cloned and expressed.

21. Process for precursor fermentation of 6-amino caproic acid starting either from 6-aminohex-2-enoic acid (6-AHEA) or from 6-amino-2-hydroxyhexanoic acid (6-AHHA), and applying at least an enzymatic step with an enzyme having α,β-enoate reductase activity towards molecules containing an α,β-enoate group and a primary amino group, in particular with an enzyme having α,β-enoate reductase activity towards 6-aminohex-2-enoic acid.

22. Process according to claim 21, characterized in that the process is performed in a microorganism wherein 6-aminohex-2-enoic acid is being formed *in vivo*.

23. Process according to claim 22, characterized in that 6-aminohex-2-enoic acid is being formed *in vivo* from solutions or slurries containing a suitable carbon source.

24. Biochemically produced 6-aminohex-2-enoic acid, having a ^{12}C versus ^{13}C versus ^{14}C isotope ratio of about the same value as occurring in environmental carbon dioxide.

25. Biochemically produced 6-amino-hexanoic acid having a ^{12}C versus ^{13}C versus ^{14}C isotope ratio of about the same value as occurring in environmental carbon dioxide.

26. ε-Caprolactam produced from biochemically produced 6-aminohex-2-enoic acid or 6-amino-hexanoic acid, and having a ^{12}C versus ^{13}C versus ^{14}C isotope ratio of about the same value as occurring in environmental carbon dioxide.

27. Nylon-6 and other derivatives produced from any of the biochemically produced products of claims 24 or 25, or from ε-caprolactam according to claim 26, and having a ^{12}C versus ^{13}C versus ^{14}C isotope ratio of about the same value as occurring in environmental carbon dioxide.